

**Listing of Claims.**

Please amend the claims as shown below by deleting the material indicated by strike-through and adding the underlined material. This listing of claims will replace all prior versions of the claims in this application.

1. (Currently Amended) A method for the purification and recovery of encysted protozoa, comprising separating the encysted protozoa from a suspension comprising the encysted protozoa by a salt flotation process wherein the salt comprises a salt selected from the group consisting of sulfates, phosphates, ~~nitrate~~s, acetates of ammonium, sodium, potassium, calcium, or magnesium, ~~or zinc~~, hydrogen-bonded organics, the salts of guanidine, and ~~or~~ mixtures thereof, ~~or a gas flotation process~~.

2. (Currently Amended) The method of claim 1, wherein ~~separating the encysted protozoa is accomplished by a~~ salt ~~sodium sulphate~~ flotation process which comprises:

preparing an admixture comprising the encysted protozoa and the salt ~~sodium sulphate~~;

centrifuging the slurry and recovering a supernatant therefrom;

forming a dilution of the supernatant and centrifuging the dilution; and

recovering the concentrate from the centrifuged dilution.

3. (Currently Amended) The method ~~mixture~~ of claim 2, further comprising:

homogenizing the admixture by high intensity homogenization.

4. (Currently Amended) The method of claim 2, wherein the salt ~~sodium sulphate~~ is present in the admixture in an amount from about 3 to about 30 weight percent.

5. (Original) The method of claim 2, wherein the specific gravity of the dilution is less than the specific gravity of the encysted protozoa.

6. (Original) The method of claim 2, wherein the concentrate comprises from about  $1 \times 10^4$  to about  $1.5 \times 10^6$  encysted protozoa/ml.

7. (Currently Amended) The method of claim ~~40~~ 4, wherein ~~separating the encysted protozoa is accomplished by~~ the gas flotation process which comprises:

adjusting the suspension to a pH sufficient to affect adhesion between bubbles of the gas in the suspension and the encysted protozoa;

conditioning the pH adjusted suspension by adding a sufficient amount of a surface active agent compound to selectively coat particles in the suspension and a sufficient amount of heteropolar compound to produce a stable froth;

passing the conditioned suspension through at least one gas flotation cell; and

recovering the encysted protozoa from the gas flotation cell.

8. (Original) The method of claim 7, wherein the gas is air.

9. (Currently Amended) The method of claim 8, wherein the adjusted pH of the suspension [pH] is about 2.5 to about 3.5.

10. (Currently Amended) The method of claim 7, wherein the surface active agent compound comprises a compound selected from the group consisting of a sodium salt of long-chain alkyl hydrogen sulfate, a quaternary ammonium compound, a blend of a fatty ammonium acetate and 2-ethylhexanol, an ester/amide compound, an alkyloxy polyethylenoxyethanol, and or mixtures thereof.

11. (Currently Amended) The method of claim 7, wherein the heteropolar compound comprises a compound selected from the group

consisting of amyl alcohols, butyl alcohols, terpinols, cresols, and ~~or~~ mixtures thereof.

12. (Original) The method of claim 7, wherein the glass flotation cell has a gas rate from about 0.25 to about 1.1 volume of gas per volume of suspension per minute.

13. (Original) The method of claim 7 wherein the gas flotation cell comprises at least two serial gas flotation units.

14. (Original) The method of claim 13, wherein the at least two units comprise different gas flow rates.

15. (Currently Amended) A method for the sporulation of oocysts, comprising:

forming an aqueous suspension of the oocysts with water and hydrogen peroxide, wherein the hydrogen peroxide is present in an amount sufficient to eliminate unwanted microbiological growth; and

~~aerating the aqueous suspension to sporulate~~  
sporulating the oocysts.

16. (Currently Amended) The method of claim 15, wherein the aqueous suspension is incubated ~~aerated~~ for a time period greater than about 40 hours such that the aqueous suspension during incubation ~~aeration~~ has a dissolved oxygen level greater than about 80% of the saturation level at a temperature of about 22°C to about 32°C and with an agitation level sufficient to adequately suspend all the solids.

17. (Original) The method of claim 15, wherein the aqueous suspension comprises an oocyst concentration of about  $10^4$  to about  $10^6$  oocysts/ml and an initial hydrogen peroxide concentration of about 1,000 to about 20,000 mg/l.

18. (Currently Amended) A method for the purification, recovery and sporulation of oocysts, comprising:  
separating the oocysts from a first suspension comprising the oocysts;  
and  
sporulating the separated oocysts by the method of claim 15 44.

19. (Currently Amended) The method of claim 46 48 wherein the oocysts are selected from the group consisting of oocysts from *Eimeria maxima*, *Eimeria mitis*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria necatrix*, *Eimeria praecox*, and combinations thereof.

20. (Currently Amended) The method of claim 47 48 wherein the salt ~~separating the oocysts is accomplished by a sodium sulfate~~ flotation process which comprises:

preparing an admixture comprising the oocysts and the salt sodium sulphate;

centrifuging the slurry and recovering a supernatant therefrom;

forming a dilution of the supernatant and centrifuging the dilution; and  
recovering the concentrate from the centrifuged dilution.

21. (Original) The method of claim 18, wherein separating the oocysts is accomplished by a gas flotation process which comprises:

adjusting the first suspension to a pH sufficient to affect adhesion between bubbles of the gas in the suspension and the encysted protozoa;

conditioning the pH adjusted suspension by adding a sufficient amount of a surface active agent compound to selectively coat particles in the suspension and a sufficient amount of a heteropolar compound to produce a stable froth;

passing the conditioned suspension through at least one gas flotation cell; and

recovering the encysted protozoa from the gas flotation cell.

22. (Currently Amended) The method of claim 18 24, further comprising:

adding a bleaching agent to the sporulated oocysts in an amount sufficient to inactivate residual microorganisms and eliminate residual organic matter; and

bleaching the sporulated oocysts.

23. (Original) The method of claim 22, wherein the bleaching is conducted concurrently with the sporulation, and the bleaching agent is hydrogen peroxide.

24. (Original) The method of claim 22, wherein the bleaching agent is sodium hypochlorite present in an amount from about 5,000 to about 10,000 parts per million free available chlorine, ozone present in an amount up to about 3% in air, or combinations thereof.

25. (Original) The method of claim 22, further comprising washing the bleached oocysts by cross-flow membrane filtration to decrease the residual bleaching agent concentration to an acceptable level.

26. (Currently Amended) The method of claim 47 25, wherein ~~the bleaching agent is sodium hypochlorite present after washing in a concentration sufficient to suppress residual microbial growth, and the~~ bleached and washed oocyst suspension has a concentration from about  $1 \times 10^6$  to about  $2.5 \times 10^6$  oocysts/ml, a maximum solids size of less than about 200 microns, and a salt ~~sodium sulfate~~ content of less than about 0.9 percent.

27. (Original) The method of claim 26, further comprising:  
concentrating the bleached and washed oocysts into a sterile concentrate;

combining sterile concentrates of one or more species of oocysts into a combined concentrate; and

packaging the combined concentrated under sterile conditions.

28. (New) The method of claim 1, wherein the encysted protozoa are *Eimeria* oocysts.

29. (New) The method of claim 28, wherein the *Eimeria* oocysts are selected from the group consisting of oocysts from *Eimeria maxima*, *Eimeria mitis*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria necatrix*, *Eimeria praecox*, and combinations thereof.

30. (New) A method for the purification and recovery of encysted protozoa, comprising separating the encysted protozoa from a suspension comprising the encysted protozoa by a salt flotation process wherein the salt comprises a salt selected from the group consisting of sodium sulfate, potassium sulfate, magnesium sulfate, sodium phosphate, potassium phosphate, magnesium phosphate, sodium acetate, potassium acetate, magnesium acetate, and mixtures thereof.

31. (New) The method of claim 30, wherein the salt comprises sodium sulfate.

32. (New) The method of claim 30, wherein the salt comprises magnesium sulfate.

33. (New) The method of claim 30, wherein separating the encysted protozoa is accomplished by a salt flotation process which comprises:  
preparing an admixture comprising the encysted protozoa and the salt;  
centrifuging the slurry and recovering a supernatant therefrom;  
forming a dilution of the supernatant and centrifuging the dilution; and  
recovering the concentrate from the centrifuged dilution.

34. (New) The method of claim 33, further comprising:  
homogenizing the admixture by high intensity homogenization.

35. (New) The method of claim 33, wherein the salt is present in the  
admixture in an amount from about 3 to about 30 weight percent.

36. (New) The method of claim 33, wherein the specific gravity of the  
dilution is less than the specific gravity of the encysted protozoa.

37. (New) The method of claim 33, wherein the concentrate  
comprises from about  $1 \times 10^4$  to about  $1.5 \times 10^6$  encysted protozoa/ml.

38. (New) The method of claim 30, wherein the encysted protozoa are  
*Eimeria* oocysts.

39. (New) The method of claim 38, wherein the *Eimeria* oocysts are  
selected from the group consisting of oocysts from *Eimeria maxima*, *Eimeria*  
*mitis*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria necatrix*,  
*Eimeria praecox*, and combinations thereof.

40. (New) A method for the purification and recovery of encysted  
protozoa, comprising separating the encysted protozoa from a suspension  
comprising the encysted protozoa by a gas flotation process.

41. (New) The method of Claim 15, further comprising aerating the  
aqueous suspension.

42. (New) The method of claim 15, wherein the oocysts are *Eimeria*  
oocysts.

43. (New) The method of claim 42, wherein the *Eimeria* oocysts are selected from the group consisting of oocysts from *Eimeria maxima*, *Eimeria mitis*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria necatrix*, *Eimeria praecox*, and combinations thereof.

44. (New) The method of Claim 15, wherein the aqueous suspension is incubated for a time period up to about 72 hours.

45. (New) The method of Claim 15, wherein the aqueous suspension is incubated for a time period between about 40 and 72 hours.

46. (New) The method of Claim 18, wherein the oocysts are *Eimeria* oocysts.

47. (New) The method of claim 18, wherein separating the oocysts is accomplished by a salt flotation process.

48. (New) The method of claim 20 or claim 47, wherein the salt comprises a salt selected from the group consisting of sodium sulfate, potassium sulfate, magnesium sulfate, sodium phosphate, potassium phosphate, magnesium phosphate, sodium acetate, potassium acetate, magnesium acetate, and mixtures thereof.

49. (New) The method of claim 48, wherein the salt comprises sodium sulfate.

50. (New) The method of claim 48, wherein the salt comprises magnesium sulfate.

51. (New) The method of claim 22, wherein the bleaching agent is hydrogen peroxide.

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